

Application Number: 09/686,880  
Filing Date: October 12, 2000  
Attorney Docket Number: 06999.0009-00000

**PROPOSED AMENDED CLAIMS**

42. (amended) A method for generating a culture that is purified or enriched in neural progenitor cells, comprising:

(i) introducing into a pluripotent cell a selectable marker that is differentially expressed in neural progenitor cells compared with its expression in other cells, wherein neural progenitor cells constitute a sub-set of the cells obtainable from the pluripotent cell, and wherein expression of the selectable marker is under the control [operatively linked to expression] of a Sox gene promoter;

(ii) culturing the pluripotent cell *in vitro* to induce differentiation of the pluripotent cell into a neural progenitor cell or into a mixture of cells including neural progenitor cells, or to induce preferential survival, in a mixed culture of cells, of neural progenitor cells; and

(iii) selecting for neural progenitor cells according to differential expression of the selectable marker introduced in step (i).

44. (previously presented) A method according to Claim 42 wherein the pluripotent cell is selected from embryonic stem (ES) cells, embryonic germ (EG) cells, embryonic carcinoma (EC) cells, a primary culture of fetal cells, a primary culture of post-natal cells, and a primary culture of adult cells.

45. (previously presented) A method according to Claim 42 comprising genetically modifying pluripotent cells by deleting, mutating, substituting or adding genes in said pluripotent cells in order (i) to assay gene function in neural progenitor cells, or (ii) to render selected cells more suitable for transplantation, or both.

Application Number: 09/686,880  
Filing Date: October 12, 2000  
Attorney Docket Number: 06999.0009-00000

46. (previously presented) A method according to Claim 42 further comprising:

- (iv) introducing into the pluripotent cell a second selectable marker that is differentially expressed in cells of a selected sub-lineage compared with its expression in other cells, wherein cells of the selected sub-lineage are formed by differentiation of neural progenitor cells; and
- (v) when a culture of neural progenitor cells has been obtained, allowing or inducing differentiation of the cells and selecting for cells that express the second selectable marker.

47. (previously presented) A method according to Claim 42 wherein the selectable marker is introduced into the pluripotent cell by targeted integration or random gene trap integration so as to be operatively coupled to a gene that is differentially expressed in neural progenitor cells.

48. (previously presented) A method according to Claim 42 wherein the selectable marker is introduced into the pluripotent cell via random integration of a transgene in which the selectable marker is operatively coupled to a gene that is differentially expressed in neural progenitor cells.

49. (previously presented) A method according to Claim 42 wherein the pluripotent cell is an ES, EG or EC cell and the method comprises forming an embryoid body in step (ii), or otherwise inducing differentiation of the cells.

50. (previously presented) A method according to Claim 49 wherein the differentiated cells are dissociated so as to form a culture comprising of individual cells.

Application Number: 09/686,880  
Filing Date: October 12, 2000  
Attorney Docket Number: 06999.0009-00000

51. (previously presented) A method according to Claim 49 wherein differentiated cells of an embryoid body are dissociated using a protease, such as trypsin.

53. (Cancelled)

54. (previously presented) A method according to Claim 42 wherein the Sox gene is selected from Sox 1, Sox 2 and Sox 3.

58. (previously presented) A method according to Claim 42 wherein the selectable marker is an antibiotic resistance gene and the method comprises culture in the presence of antibiotic.

64. (previously presented) A method of preparing a neural progenitor cell or a differentiated progeny thereof for storage, comprising obtaining the cell in a method according to Claim 42 and freezing the cell in the presence of a cryoprotectant.

65. (previously presented) A method of generating purified neuron cells, comprising obtaining a culture of neural progenitor cells using the method of Claim 42 wherein the selectable marker is differentially expressed in cells that express a Sox gene, and culturing the progenitor cells obtained in the presence of medium suitable for differentiation of the progenitor cells into neuron cells.

66. (Amended) A method for generating a culture that is purified or enriched in neural progenitor cells, comprising:

(i) introducing into a pluripotent cell a selectable marker that is differentially expressed in neural progenitor cells compared with its expression in other cells, wherein neural progenitor cells constitute a sub-set of the cells obtainable from the pluripotent cell, and wherein expression of the selectable marker is under the control [operatively]

Application Number: 09/686,880  
Filing Date: October 12, 2000  
Attorney Docket Number: 06999.0009-00000

linked to expression] of a promoter of a gene that is differentially expressed in neural progenitor cells,

(ii) culturing the pluripotent cell *in vitro* to induce differentiation of the pluripotent cell into a neural progenitor cell or into a mixture of cells including neural progenitor cells, or to induce preferential survival, in a mixed culture of cells, of neural progenitor cells; and

(iii) selecting for neural progenitor cells according to differential expression of the selectable marker introduced in step (i).

67. (previously presented) A method according to Claim 66 wherein the pluripotent cell is selected from embryonic stem (ES) cells, embryonic germ (EG) cells, embryonic carcinoma (EC) cells, a primary culture of fetal cells, a primary culture of post-natal cells, and a primary culture of adult cells.

68. (previously presented) A method according to Claim 66 comprising genetically modifying pluripotent cells by deleting, mutating, substituting, or adding genes in said pluripotent cells in order to assay gene function in neural progenitor cells, or render selected cells more suitable for transplantation, or both.

69. (previously presented) A method according to Claim 66 further comprising:

(1) introducing into the pluripotent cell a second selectable marker that is differentially expressed in cells of a selectable sub-lineage compared with its expression in other cells, wherein cells of the selected sub-lineage are formed by differentiation of neural progenitor cells; and

(2) when a culture of neural progenitor cells has been obtained, allowing or

Application Number: 09/686,880  
Filing Date: October 12, 2000  
Attorney Docket Number: 06999.0009-00000

inducing differentiation of the cells and selecting for cells that express the second selectable marker.

70. (previously presented) A method according to Claim 66 wherein the selectable marker is introduced into the pluripotent cell by targeted integration or random gene trap integration so as to be operatively coupled to a gene that is differentially expressed in neural progenitor cells.

71. (previously presented) A method according to Claim 66 wherein the selectable marker is introduced into the pluripotent cell via random integration of a transgene in which the selectable marker is operatively coupled to a gene that is differentially expressed in neural progenitor cells.

72. (previously presented) A method according to Claim 66 wherein the pluripotent cell is an ES, EG, or EC cell and the method comprises forming an embryoid body in step (ii), or otherwise inducing differentiation of the cells.

73. (previously presented) A method according to Claim 72 wherein the differentiated cells are dissociated so as to form a culture substantially of individual cells.

74. (previously presented) A method according to Claim 72 wherein differentiated cells of an embryoid body are dissociated using a protease, such as trypsin.

75. (previously presented) A method according to Claim 66 wherein the selectable marker is an antibiotic resistance gene and the method comprises culture in the presence of antibiotic.

NOV 17 2003 15:45 FR FINNEGAN HENDERSON 617 452 1666 TO 917037467043# P.07

Application Number: 09/686,880  
Filing Date: October 12, 2000  
Attorney Docket Number: 06999.0009-00000

Application Number: 09/686,880  
Filing Date: October 12, 2000  
Attorney Docket Number: 06999.0009-00000

76. (previously presented) A method according to Claim 66 wherein expression of the selectable marker is operatively linked to expression of a gene selected from the group consisting of Pax 3, delta-1, Mash-1, Math-4a, Pax 6,  $\beta$ 3-tubulin, synapsin-1, MAP2/tau, GFAP, GABA, and islet -1/2.

77. (previously presented) A method of preparing a neural progenitor cell or a differentiated progeny thereof for storage, comprising obtaining the cell in a method according to Claim 66 and freezing the cell in the presence of a cryoprotectant.

78. (amended) A method of generating purified neurons, comprising obtaining a culture purified in respect of neural progenitor cells [progenitors], using the method of Claim 66 wherein the selectable marker is differentially expressed in neural progenitor cells and culturing the progenitor cells [progenitors] obtained in the presence of medium suitable for differentiation of the progenitor into neurons.